**Biotechnology and Genetic engineering**

**Dr. Abeer Fauzi Murad Lecture (3-4)**

**Fermentation**

**What is the fermentation?**

Fermentation is a metabolic process, which converts carbohydrates to alcohols, organic acids or gases by the activity of enzymes of microbial origin. Microbes involved in fermentation process: Bacteria and Fungi.

The process of anaerobic respiration in the muscle cells of animals during exercise, which produce lactic acid, is also a type of fermentation. The technique of fermentation was very ancient in origin Egyptians and Sumerians had the knowledge of the technique of converting starchy grains to alcoholics

For a microbiologist, the word ‘fermentation’ means many processes such as:

***1-A method of mass cultivation of microbes under aerobic or anaerobic conditions***

***2-Any biological process occurs in the absence of oxygen***

***3-Spoilage of food by microbial activity***

***4-Production of alcoholic beverages, organic acids, antibiotics or biopolymers***

***5-Partial oxidation of carbohydrates***

In the biochemical sense the term fermentation, refer to a metabolic process in which organic compounds (particularly carbohydrates) are broken down to release energy without the involvement of a terminal electron acceptor such as oxygen.

The term fermentation can be also be applied to any industrial process that produce a material that is useful to human and if the process depends on the activity of one or more M.Os. This processes known as industrial fermentation, are usually carried out on large scale and in a vessels in which the organisms are normally grow on liquid media.

**What is industrial fermentation?** The intentional use of fermentation technology for the large-scale production of microbial biomass or metabolites is called industrial fermentation. Fermented products have immense use in food, medicine and other industries. Modern industrial fermentation units use genetically engineered microbes for the rapid production of desired metabolites.

***A vast range of materials is produced by industrial fermentations and these includes:***

1- Organic chemicals used as fuels, food additives (yeast extract from yeast cells produced as bio product of brewing industry), antibiotics and enzymes for use in the food and other industries. Vinegar is an example of a food additive produced by industrial fermentation.

2-Food produced on a large scale as a result of the activities of microorganisms. E.g. cheese, yoghurt and bread.

3-Production of alcoholic beverages.

Materials produced by industrial fermentation can be divided in to four groups:-

1 -Microbial biomass such as organisms produced on a large scale for the extraction of protein(single cell protein)that can be used as a part of the of the human diet.(Quorn is an example of a single cell protein produced by the fungus *Fusarium* *graminearum* m the mycoprotien purified from fungi is used as food and incorporated with a high dishes, Mushroom production another process that can be considered as industrial fermentations.

2-Microbial enzyme

3-Microbial metabolite:

A-Primary metabolite products: microorganisms produce it's during the log phase (trophophase) many essential materials very important in it is growth such as amino acids, nucleic acids, nucleotides, proteins, lipids, carbohydrates.

B-Secondary metabolite products: microorganisms produce it's during the stationary phase (idiophase) these substance have no relation with the growth processes these product used in pharmaceutical manufacturing industry.

4-Bioconversions: M.Os cells were used for modifying special compounds been added previously to the culture media, to other compounds close to it (from the chemical level) but its more valuable on the commercial level. The bioconversion include hydration, oxidation, hydroxylation…ets.

The bioconversion characterized from the ordinary chemical conversion by the specialization and that can work with in low temperature and with the absence with the heavy metals that cause pollution for example production of acetic acid from alcohol solutions it produce by oxidation of ethanol by Acetobacter.

**Increasing the production of primary metabolites:**

While the cell cannot produce large scale of primary metabolites because of cellular control; there is some way to increase the production:

1-Genetic modification: the purpose of this to get high productive strains

2-Modification of culture components: this can lead to change the permeability of cell membrane or to increase the production.

**Control and regulation of secondary metabolites process:**

The control over this process can be done by many way:

1-By controlling the chromosomes that determination the genetic information which responsible for production and creation the enzymes which taking a part in its production.

2-Controlling the energy quality represented by ATP, ADT, AMP

3-Controlling the culture material.

General features of secondary metabolites:

1-These materials have no relation to the growth processes especially the log phase, that is why it does not show up in idio phase or reproductive phase.

2- These materials are produced by the cells within stationary phase and sometimes produced by cells that are within very low growth rate cells.

3- Most of the secondary metabolites materials are either antibiotics or pharmacological materials or toxins extrinsic enzymes. some of these materials have no physiological action regarding the M.Os but it has a basic action in some of high specialized biological activity.

4- Most of the secondary metabolites materials are secreting out of the cells.

5- The secondary metabolites materials are produced in high quantity in compare with primary metabolites materials.

6-These materials are produced by a specials group of M.Os or plants

7-The creation paths that by it these materials produced are very limited and most of it have relation with the primary metabolites materials, it is coming from specific materials during the paths.

**What is a fermenter?**

The fermenter: is a tightly sealed reaction chamber in which a controlled reaction can take place. It keeps any contaminants (biotic and abiotic alike) from disturbing or even spoiling the enclosed reaction mechanism.

The heart of industrial fermentation is a ‘Fermenter Fermenter is type of bioreactor.

Fermenter: a system provided with controlled environmental conditions for the growth of microbes in liquid culture and production of specific metabolites.

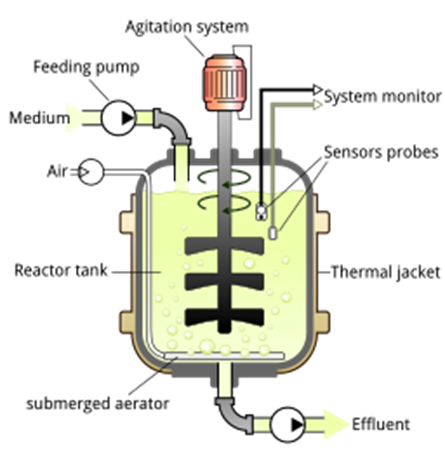
A-It is a device in which the microbes are cultivated and motivated to form the desired products

B- It is containment system to provide the accurate environment for the optimum growth and metabolic activity of the microbes.

C- Fermenter prevents the entry and growth of contaminating microbes from outside.

D- Fermenter: containment system for the cultivation of prokaryotic cells (bacteria) and fungi

E- Bioreactor: containment system for the cultivation of mammalian or insect cells

Fermenter (fig 1)

**What are the different types of fermentation process / methods?**

There are three types of industrial fermentation processes based on the methods of fermentation and types of fermenters

***1-Batch fermentation***

***2-Continuous fermentation***

***3-Fed-batch fermentation***

**1-Batch Fermentation:**

1- Microorganism is inoculated into a fixed volume of medium

2- As the growth takes place, the nutrients are consumed and the product of growth accumulates in the fermenter

3-Product of growth may be of two types: (a) Biomass and (b). Metabolites

4-The nutrient environment in the fermenter is continuously changed

5-This change in the environment in the fermenter will enforce change in the metabolism of cells

6-This also results in the cessation of cell multiplication

7- Cessation of growth is due to the scarcity of nutrients and accumulation of metabolites

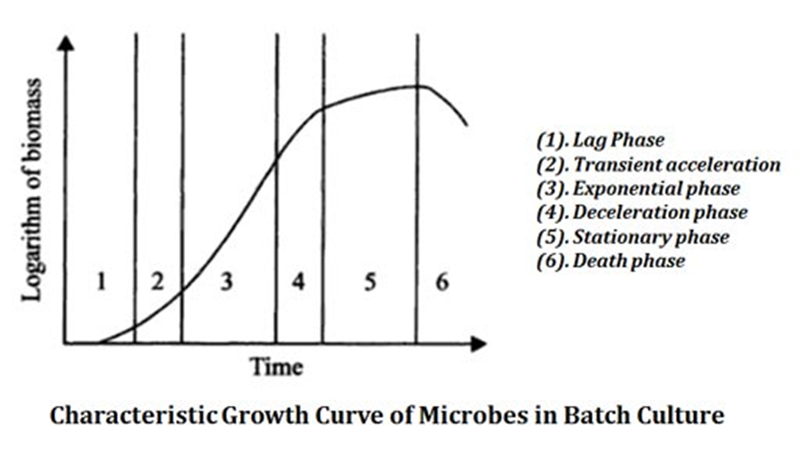
8-Once the microbes reached the stationary phase they start to accumulate the metabolites

9-Metabolites are extracted from the fermenter by downstream processes

10-After the fermentation is over, the residues are taken out from the fermentation tank, and the vessel is then cleaned and sterilized before next batch of fermentation

11-Thus in batch fermentation, the large scale production is done as separates ‘batches’

12-Microbes in the batch culture show the following pattern of growth with distinct phases



(1)Log phase: initial phase, no apparent growth of microbes, they adapt to the environmental conditions

(2)Transient acceleration: the inoculum begins to grow slowly.

. (3)Exponential phase: microbial growth proceeds at the maximum possible rate

(4)Deceleration phase: decline in the growth rate of microbes

(5)Stationary phase: no overall growth rate (death of the cells equals to the division of cells). Most of the secondary metabolites are produced in this phase

(6)Death phase: no growth at all, cells starts to die and the population size decreases. Usually the fermentation stops before the death phase.

The batch cultures characterized by it is contain limited quantity of nutrient materials the inoculate has been added at the beginning of the fermentation and under changeable circumstances like the concentration of the growth suppressing nutrient material and pH may change during the fermentation and the side products increase more as time pass, the air supply by shaking the culture with the vibratory.

**(2). Continuous Fermentation:**

(a) Here the exponential growth rate of the microbes is maintained in the fermenter for prolonged periods of time in by the addition of fresh media are regular intervals

(b) Microbes reach the exponential growth rate and continue as such due to the availability of nutrients

(c) The exponential growth rate of microbes continues till the vessel becomes completely filled in the cells

(d) Continuous fermenter possesses devices for the collection of overflow from the vessel

(e) The metabolite or the product of fermentation is extracted for the overflow by downstream processing

(f) Thus unlike batch fermentation, in continuous fermentation, the fermentation process never stops in between and it continues to run for a long period of time with the addition of nutrients and harvesting the metabolites at regular intervals.

Continuous Fermentation characterized by continuous moving out from and out of the system, the metabolism reaction are in stable states ,the air changes should be continuous while the side products can be removed from time to time to keep the volume constant and these system include:

1-chemostate :regulation of the culture can be done by adding one of the basic materials and by computed levels by the sensors.

2-biostate:regulation of the entrance and extranet of the nutrient material by the measuring some of side products like CO2 and the use of O2 .

3-Tubidostate: regulation depending on cells measuring with in the culture media by indirect principles , the cultures are connected to a light cell and then measuring the darkness and as a darkness increase the machine will give a signal to enter more quantity of the sterilized culture and remove equal quantity from the fermentation media, and if the cells concentration decrease the passage will close to prevent the passage of the cells out to enable it to increase its concentration and reproduce.

**Similarities and difference between Batch Fermentation and Continuous Fermentation Process:**

*The Similarities between Batch Fermentation and Continuous* Fermentation Process:

1-Both are industrial fermentation methods for the large scale production.

2-Both methods can be used for the production of microbial biomass or products.

3-Both run under controlled environmental conditions

4-The mechanical components of fermenter is almost similar in both types.

*The difference between Batch Fermentation and Continuous Fermentation Process:*

|  |  |
| --- | --- |
| Continuous Fermentation | Batch Fermentation |
| 1-It is an open system  2- Setup is changed from outside during the fermentation process.  3- The process is not stopped for the collection of the products, but it is continuously taken out from the fermenter.  4- Nutrients are added many times (in the beginning and in between the fermentation process).  5- More control on the growth and production.  6- Environmental condition in the fermenter will be kept constant.  7- Turnover rate will be high.  8- Nutrients in the fermenter are utilized in relatively fast rate.  9- Optimum or exponential growth rate of microbes is maintained in the fermenter.  10- Contents of the fermenter are NOT removed for the isolation of products. Products are extracted from the overflow from the fermenter.  11- No such washing step required since continuous addition of nutrients and microbes are performed.  12- Smaller size fermenter is required, since the yield is very high.  13- More closer to the natural environment. | 1-It is a closed system  2- Setup is not changed from outside once the fermentation is started  3- The process is stopped once the product is formed.  4- Nutrients are added only once (in the beginning) and not added in between the fermentation process.  5- Less control over the growth of the microbes and the production of desired products.  6- Environmental conditions in the fermenter will not be constant.  7- Turnover rate (conversion of the substance to desired product) is less.  8- Nutrients in the fermenter are utilized in relatively slow rate.  9- Microbes in the fermenter show lag, log and stationary phases.  10- 0 Contents of the fermenter are removed after the fermentation process for the isolation of products.  11- The fermenter is washed and cleaned before the next step of fermentation.  12- Relatively larger size fermenters are used.  13- Less close to the natural environment. |

**Many ways use to attempt some conversion to the system to increase its efficiency:**

1-adding of filters to the culture of the fermentation to decrease the passage out of the cells and that will increase the concentration.

2-using multi steps cultures media by using more than one fermenting agent and application of different fermentation circumstance with n different vessels.

**Determination of the culture media components and controlling the ideal ecological circumstances for the production:**

1-The culture media: the culture media should contain the basic nutrient components that enable the growth of M.Os strains like( Nitrogen, Carbon and Phosphor) and also (corn sugar, mollase, starch, cellulose).

2-The air exposing: most of the fermentation processes are aerobic that is why it is need the presence of oxygen

3-The foam control: fast air exposing and mixing lead to the formation of foam in the culture media and this one of the problems that face most of the fermentation process because of the presence of protein materials. The aggregation of foam in the air out and in windows lead to exposure the fermentation to the pollution, we can control the foam formation by using anti foam.

4-Controlling the pH: the pH have a big effect on the fermentation process, metabolisms activity for product formation require optimum rang for pH

5-Tempreture: temperature must regulate for media to achieve perfect production of the required material. The fast growing for the M.Os sometimes leads to generate rising in temperature which lead to reducing the enzyme reactions, so we should decrease and regulate culture media temperature by passing cold water in coils submerged in the media inside the fermenter or inside the chamber surround the fermenter.

**3-Fed-batch fermentation**

A-It is a modified version of batch fermentation

B-Here the substrate is added in increments at different times throughout the course of fermentation

C-Periodical addition of substrate keeps the prolonged log and stationary phase of the microbes in the fermenter

D-These results in the rapid increase of biomass

E-Consequently, increased production of metabolites can be achieved in the stationary phase

F-Thus fed-batch technique is an improved version of fermentation by avoiding the disadvantages of batch and continuous fermentation techniques.

**Solid-state fermentation**

*SSF :* Defined as the growth of the micro-organisms on (moist) solid material in the absence or near-absence of free water

Solid substrate fermentation: Processes in which the substrate itself acts as carbon/energy source, occurring in the absence or near-absence of free water

Economically and industrially important advantages of SSF

* Lower capital and recurring expenditure
* Lower waste water output / less water need
* Reduced energy requirement
* Simplicity
* Simpler fermentation media
* Absence of rigorous control of fermentation parameters
* Easier aeration
* Economical to use even in smaller scales
* Applicability of using fermented solids directly
* Storage of dried fermented matter
* Lower cost of downstream processing

**Factors needs to consider**

1-Biological factors: metabolic process and reproduction

2- Physico-chemical factors: related with the transport phenomena of momentum, energy and mass transfer

These the two factors will have impact on (Biological factors and

Physico-chemical factors)

**1-strains selection,**

**2-design of medium for fermentation,**

**3-substrate conditioning,**

**4-control criteria,**

**5-design of reactors.**

**Physico-chemical factors include:**

1-water activity and moisture content of the substrate.

2-temperature and heat transfer.

3-pH. (it determines the enzyme activities)

4-aeration.

5-nutrient diffusion. It affects the nutrient concentration and regulates the actions of enzymes over the solid substrate

6-Mixing : It helps heat removal, gas exchange, water content, uniformity and influences the process conditions.

**Dis advantages of SSF**

1. The fermentation process is so slow because is take so long time
2. Difficulty in controlling the production circumstance like pH, Temp, CO2, O2 and humidity
3. The fermentation process are limited to certain types of M.Os like fungi which grow with in low water media
4. High Temp and sometimes difficult to remove
5. Exposed to pollution
6. There is no ability to expand the production in this fermentation
7. Difficulty to control the humidity in these fermentation
8. Difficulty in recording the exact information about the fermentation processes like O2,CO2 level and the determination of the formed biological mass quality

SSF applications:-

1-Production of acids like lactic acid

2- Production of biological mass

3- Production of trade enzymes

4- Production of mushroom

5- Production of biological gas like methane

**Selection of strain that used in biotechnology**

The selection of strain depend on production capability and the economic cost, there are many features must be present in M.Os to be select:

1-Prefer to select isolates that able to grow on cheap and available materials the whole year.

2- Selection of isolates that need high temperature for their growth rate are high and it dose the bioconversion with high rate and that help to decrease the cooling cost.

3-Selection of isolates with high production rate and low side product to facilities the separation, purification that done after the fermentation

4-Selection of stable strains that not show the polymorphism phenomena to facilitate the detection of pollution

5- Selection of genetically stable strain and the strain that can be manipulate genetically to developed strain productions

6-Selection of strains that does not produce toxins under any growth conditions

7-Selection of strains that can kept for a long time.